Selection and Turnover of Coelenterate Nematocysts in Some Aeolid Nudibranchs

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INTRODUCTION

The storage of coelenterate nematocysts by aeolid nudibranchs is well-known (Wright, 1859; Grosvenor, 1903; Cuénot, 1907; Glaser, 1910; Graham, 1938; Kepner, 1943; Edmunds, 1966; Thompson & Bennett, 1969, 1970; and Mariscal, 1974); however, the mechanisms involved and the dynamics of the system are poorly understood. Graham (*op. cit.*) and Kepner (*op. cit.*) described the pathway of nematocysts into the cnidosacs and their uptake by the cnidosac cells. Kepner found that *Aeolis* (= *Cratena*) *pilata* (Gould, 1870) cnidosac cells took up all nematocyst types from the hydroid *Pennaria tiarella* (Ayres, 1854), but maintained only the microbasic mastigophores while digesting the other types. Edmunds (*op. cit.*) found that different aeolid species varied in their selectivity of nematocyst types from species that maintained only one type to species that held the entire cnidom of their cnidarian prey. Grosvenor (*op. cit.*) reported that the nematocyst complement of *Rizzolia* (= *Cratena*) *peregrina* (Deshayes, 1838) was almost entirely replaced a month after shifting the nudibranch to a new hydroid species.

One of us (RMD) spent the spring of 1975 at the Duke Marine Laboratory at Beaufort, North Carolina. During this period *Cratena pilata* were feeding on *Tubularia* spp. instead of *Pennaria* as reported by Kepner (1943). The *Cratena* feeding on *Tubularia* had primarily stenoteles in their cnidosacs versus the microbasic mastigophores present when the prey is *Pennaria*. This observation, as well as the report of different nematocyst preferences with different hydroid prey made by Grosvenor (1903), suggested that nematocyst selection in aeolid nudibranchs is a complex and dynamic phenomenon which requires further study. The purpose of this report is to describe and discuss the results of studies on nematocyst selection and turnover in the aeolid nudibranch *Coryphella verrucosa* (Sars, 1829).

MATERIALS and METHODS

The studies reported here were conducted in the Zoology Department of the University of New Hampshire, Durham, New Hampshire during the summer of 1975. Nudibranchs and their cnidarian prey were collected from floats and pier pilings in Portsmouth Harbor, New Hampshire and benthic communities in Gosport Harbor, Isles of Shoals (43°59′N; 70°37′W) off the New Hampshire coast. Animals were maintained in a recirculating seawater system at 13° C or in an incubator at 5° C. Survival was greater at 5° C, so all experiments were run at this temperature.

Three types of observations were made during the study. First, a series of aeolid species was collected along with their coelenterate prey. The nematocyst complement of each individual was determined by removing a single ceras with fine pointed forceps, squashing it between a glass slide and coverslip and identifying nematocysts extruded from the ceras tip using the key in Mariscal (1974). The nematocyst complement of each nudibranch was compared to that of the prey species on which it was found in the field.

A cnidosac regeneration experiment was conducted using *Coryphella verrucosa*. This species was used because it is common and eats a variety of coelenterate prey. The experiment consisted of removing the tips of all the cerata anterior to the heart and then allowing the test subjects to feed on the hydroid *Hydractinia echinata* Fleming, 1828. Squashes were made of 3 cerata anterior to and posterior (controls) to the heart on each of the 5 exper-

imental animals. The presence of a large number of nematocysts in ceras tip squash mounts was used as an indicator of cnidosac regeneration.

The third experiment consisted of isolating groups of 5 nudibranchs, each with previously determined nematocyst complements, with different hydroid species and monitoring by ceras-squash preparations any changes in the nematocyst complement in each group. The first 25 identifiable nematocysts were recorded in each squash preparation.

RESULTS AND OBSERVATIONS

The nematocyst complements of several aeolid nudibranch species collected on known coelenterate prey are summarized in Table 1. As is obvious from this table, many aeolid species are quite specific in their selection of nematocyst types and the type varies according to the prey species being consumed. *Cratena pilata* selects microbasic mastigophores when eating *Pennaria* (KEPNER, 1943) and steno-

Table 1

Summary of nematocyst types observed in the cnidosacs of several aeolid nudibranchs as compared with their coelenterate prey. Where more than one type was present, the dominant form is indicated by an asterisk (*).

Nudibranch species	Coelenterate Prey	Nematocysts identified in Coelenterate	Nematocysts identified in Cnidosacs
Cratena pilata	*Tubularia* spp.	stenoteles basitrichous isorhizas holotrichous or atrichous isorhizas desmonemes	stenoteles* basitrichous isorhizas
Aeolidia papillosa	*Metridium senile*	spirocysts basitrichous isorhizas microbasic mastigophores microbasic amastigophores atrichous isorhizas holotrichous isorhizas	microbasic amastigophores basitrichous isorhizas
Catriona aurantia	*Tubularia* spp.	stenoteles basitrichous isorhizas holotrichous or atrichous isorhizas desmonemes	stenoteles basitrichous isorhizas
Cuthona nana	*Hydractinea echinata*	desmonemes microbasic euryteles microbasic mastigophores holotrichous or atrichous isorhizas	microbasic mastigophores
Facelina bostoniensis	*Tubularia* spp.	stenoteles basitrichous isorhizas holotrichous or atrichous isorhizas desmonemes	stenoteles
F. bostoniensis	*Eudendrium* spp.	microbasic euryteles microbasic mastigophores	microbasic mastigophores microbasic euryteles*
Coryphella verrucosa	*Hydractinea echinata*	desmonemes microbasic euryteles microbasic mastigophores isorhizas (holotrichous or atrichous)	microbasic mastigophores* desmonemes
C. verrucosa	*Obelia geniculata*	basitrichous isorhizas	basitrichous isorhizas
C. verrucosa	*Tubularia crocea*	stenoteles basitrichous isorhizas holotrichous or atrichous isorhizas	stenoteles* basitrichous isorhizas
C. verrucosa	*Lucernaria* spp.	Microbasic euryteles atrichous isorhizas	microbasic euryteles

teles when feeding on *Tubularia*. *Coryphella verrucosa* also stores stenoteles from *Tubularia*, but selects microbasic mastigophores when feeding on *Hydractinia*, basitrichous isorhizas from *Obelia* spp. and microbasic euryteles when eating the stauromedusan *Lucernaria* sp.

There appears to be a consistent pattern of preference for certain nematocyst types in several nudibranch species when consuming the same prey species. *Cuthona nana* (Alder & Hancock, 1842) and *Coryphella verrucosa* both store microbasic mastigophores when eating *Hydractinia*, while *Coryphella verrucosa, Cratena pilata* and *Catriona aurantia* (Alder & Hancock, 1842) select stenoteles when feeding on *Tubularia* spp.

The regeneration experiment provided two significant results. Nematocysts were observed in regenerating cerata in all animals within 12 days, indicating the presence of functional cnidosacs. By this time the cnidosacs of the posterior, or control cerata, contained only microbasic mastigophores instead of the euryteles present at the start of the experiment. There had been a total replacement of nematocysts in less than 12 days.

An experiment was then performed in which a group of *Coryphella verrucosa*, collected from an area containing *Lucernaria* sp., *Obelia* sp. and *Tubularia crocea*, were isolated and fed specific hydroid species; the nematocyst complements of the nudibranchs' cnidosacs were then monitored. Table 2 summarizes the results of this experiment. In each case, the turnover time was 3 to 4 days. The ceras squashes often contained more than one nematocyst type, even after the nematocyst complement had become dominated by a new type. In the case of the *Obelia*, which has only basitrichous isorhizas, closer examination turned up some actinulae larvae of *Tubularia* among the colonies. There were no contaminating hydroids in the other setups, but the nudibranchs were not isolated from food for any period of time before the ceras squashes were made; it is likely, therefore, that other nematocysts seen were from a recent meal and had not been processed as yet.

DISCUSSION

One of the more fascinating aspects of the association between aeolid nudibranchs and coelenterates is the storage and utilization of functional nematocysts. The position of the cnidosacs at the tips of the cerata and the fact that nematocysts are ejected when the nudibranch is

Table 2

Results of experiment to determine selectivity and turnover rates of nematocysts in *Coryphella verrucosa* fed three hydroid species. The nudibranchs were found associated with the stauromedusan *Lucernaria* sp. though *Obelia* spp. and *Tubularia crocea* were also present.

Prey species	Nematocysts Observed in Cnidosacs				Minimum Time
	Start of Experiment		After eight to ten days		
	Dominant Form	Also seen	Dominant Form	Also seen	Turnover
Hydractinea echinata	microbasic euryteles		microbasic mastigophores	stenoteles[1] desmonemes	4
Tubularia crocea	microbasic euryteles	stenoteles microbasic mastigophores basitrichous isorhizas atrichous isorhizas	stenoteles	euryteles[1] microbasic mastigophores basitrichous isorhizas	4
Obelia geniculata	microbasic euryteles	stenoteles microbasic mastigophores atrichous isorhizas	basitrichous isorhizas	stenoteles	3

[1]—seen in only one specimen.

disturbed has led to the general conclusion that they must be used for defense by aeolids (Garstang, 1894; Kepner, 1943; Edmunds, 1966; Harris, 1973). In fact, there is to date only one documentation of a defensive function for nematocysts in aeolids based on experimental evidence. Allen (1976) reported finding basitrichous isorhizas in the puffed and necrotic mouth tissue of a shiner surfperch *Cymatogaster aggregata* Gibbons, 1854, that had bitten and rejected a *Hermissenda crassicornis* (Eschscholtz, 1831).

Several authors (Kepner, 1943; Edmunds, 1966; Thompson & Bennett, 1969) have suggested that the selection of specific nematocyst types is an adaptation to store the most effective nematocysts against predators. Edmunds (*op. cit.*) suggested that different nematocyst types were selected for in response to different predators — penetrants against fish predators and desmonemes for crustaceans.

Thompson & Bennett (1969, 1970) proposed that aeolids maintain a supply of certain nematocyst types independent of the number of different coelenterates preyed upon, stating that *Glaucus atlanticus* (Forster, 1777) and *Glaucilla marginata* (Bergh, 1868) employ *Physalia* nematocysts for defense, usually digesting those from *Porpita* and *Velella*. Grosvenor (1903) gave the first indication that different nematocysts might be incorporated from different prey by stating that the nematocyst complement could vary among individuals of the same species. The results of this study confirm Grosvenor's observations and provide evidence that at least some aeolid species incorporate specific nematocyst types with each prey species and the nematocyst type varies from prey species to prey species. Kepner (1943) reported that *Cratena pilata* stored microbasic mastigophores when feeding on *Pennaria tiarella* and we found that *C. pilata* selects stenoteles when it eats *Tubularia* spp. Mariscal (1974) stated that *Aeolidia papillosa* (Linnaeus, 1761) stored basitrichous isorhizas when feeding on *Epiactis prolifera* Verrill, 1869, while we found microbasic mastigophores and basitrichous isorhizas in specimens associated with *Metridium senile* (Linnaeus, 1767) *Coryphella verrucosa* selects 4 separate nematocyst types from 4 prey species. In regard to Thompson & Bennett's observations, the aeolids may not have been selecting against the nematocysts from *Porpita* and *Velella*, but had simply fed on *Physalia* most recently and the nematocysts from the other coelenterates were in the process of being replaced.

Several aspects of the selection mechanism appear to be of particular interest, specifically the rate of turnover and the significance of selecting a distinct nematocyst type. Grosvenor (1903) reported that the replacement in nematocyst complement after changing the prey species in *Cratena peregrina* was about 30 days. The results of this study suggest the process of replacement may occur much faster. When considering Kepner's (1943) observations that nematocysts were present in the cnidosacs of *Cratena pilata* 25 minutes after feeding and that none remained in the stomach after 35 minutes, the faster turnover rate does not seem unreasonable.

The fact that a process of replacing unused nematocysts is occurring and that the turnover rate is relatively quick suggests that there may be a limit on the length of time a nematocyst remains functional in the nudibranchs' cnidocyte analogue, the cnidosac cells. It is possible that nematocysts which are very complex cell organelles may lose their ability to fire over time. If this is the case, then selection pressure should favor a rapid turnover of nematocysts. Starved nudibranchs still contain nematocysts after several weeks, which suggests that the turnover rate varies according to the nutritional state of the nudibranch. If nematocysts are stored primarily for defensive purposes, then natural selection should favor maintaining a nematocyst complement even though its effectiveness may be decreasing over time. Therefore, while the number of functional nematocysts might decrease, a percentage will still be able to discharge, providing the aeolid with more protection than if all nematocysts had been discarded leaving none for defense. It must be kept in mind that nematocyst utilization is only one of several defensive mechanisms typically employed by aeolid nulibranchs, including cryptic coloration, autotomy of cerata, and secretions from ceratal glands (Edmunds, 1966).

Little information is available on the turnover of nematocysts in coelenterates. Bode & Flick (1976) found that nematocysts in *Hydra attenuata* (Pallas) are replaced in 7 to 9 days, so it may be that even in cnidocysts, nematocysts become nonfunctional in a relatively short period of time. The information available suggests that studies of nematocyst dynamics in coelenterates may provide some interesting insights into nematocyst biology.

There appear to be two possible mechanisms for the turnover of nematocysts in nudibranch cnidosacs. The first explanation is an extension of the mechanism for nematocyst selection (Kepner, 1943). Cnidosac cells would simply continue to engulf new nematocysts as long as they were available, digesting non-preferred types and holding preferred ones. A second possibility is that new cnidosac cells are continuously being produced at the neck of the cnidosac and older cells are sloughed off or somehow eliminated. Since starved nudibranchs maintain nematocysts much longer than fed animals, cell production may decrease when coelenterate prey, and therefore nematocysts,

were not available. The most likely explanation for the mechanism of nematocyst turnover seems to be a combination of both cell proliferation and turnover within individual cells.

An obvious question raised by this study is why a different type of nematocyst is selected from each prey genus or species if presumably the same types of nematocysts are available? Nematocysts are cell organelles with several functions in coelenterates including offense, defense and adhesion (Mariscal, 1974), but little information is available on the function of specific nematocyst types and how this might vary from species to species. The selection of distinct nematocyst types for each prey species by a nudibranch suggests a complex recognition mechanism in cnidosac cells. The fact that the same cell selectively maintains stenoteles from one prey species, microbasic mastigophores from another, basitrichous isorhizas from a third and microbasic euryteles from a fourth prey species also indicates the nematocysts from different species may not be as similar in function as their morphology might suggest. Stenoteles are superficially similar in most hydroid species, but their function and the stimuli which induce firing may vary significantly. *Cratena pilata* stores stenoteles when it feeds on *Tubularia*, but not when it eats *Pennaria* (Kepner, 1943). An alternative hypothesis might be that cnidosac cells are selecting for relative abundance of nematocyst types in any given prey species. However, this hypothesis does not account for the fact that only one type is maintained in each case and it neglects the functional explanation for why nematocysts are stored-defense (Kepner, 1943; Edmunds, 1966; Allen, 1976).

Aeolids from separate families feeding on the same prey species select the same nematocyst type; this suggests that nudibranchs occurring in the same habitat and overlapping in their prey preferences may also be subject to similar selection pressure from predators, resulting in their picking the same type. Kepner (1943) proposed that the microbasic mastigophores of *Pennaria tiarella* were more effective predator deterrents for *Cratena pilata* than the larger penetrants which appeared to be stenoteles. If Kepner is correct, then the stenoteles of *Tubularia* spp. and microbasic mastigophores in *Hydractinia* should be the most effective predator deterrents in these hydroids.

Not all aeolids show the same specificity as has been shown for most of the species discussed in this report. Edmunds (1966) found that some aeolid species did not select specific nematocyst types, but tended to have several types in the cnidosacs at any one time. Mariscal (1974) found 2 types of nematocysts in the cnidosacs of *Hermissenda crassicornis* and one type in *Aeolidia papillosa*, and *Facelina bostoniensis* (Couthouy, 1838) may store more than one nematocyst type when feeding on certain prey species.

Coryphella verrucosa is a generalist in that it eats a variety of hydroid species and stauromedusans, but individuals do concentrate their efforts on locally and temporally abundant species as predicted in ecological theory (Emlen, 1966, 1968; MacArthur & Pianka, 1966). While cnidosac cells in many aeolids store only one type of nematocyst from each prey species, they ingest all types and digest those types not maintained; this suggests that it is energetically advantageous to engulf and digest the nonpreferred nematocyst types rather than to engulf only the type to be utilized for defense. Another implication of this process is that the site of recognition and selection is within the cytoplasm rather than on the cell membrane where most chemoreception takes place.

In conclusion, the results of this study show that nematocyst utilization in aeolid nudibranchs is a dynamic and complex phenomenon which involves selection of specific types determined by the prey species consumed and that there is a rapid turnover of stored nematocysts. A number of aspects of this system would seem to offer promising avenues for further research.

SUMMARY

Observations on the nematocyst complement of several aeolid nudibranchs resulted in the findings that at least a number of aeolid species select specific nematocyst types from their coelenterate prey and that different nematocyst types are stored when different prey species are eaten.

A regeneration experiment showed that cnidosac replacement in *Coryphella verrucosa* was complete in less than 12 days at 5° C. The turnover of nematocyst types was also found to be much faster than previously reported.

Groups of *Coryphella verrucosa* were fed different hydroid prey and the turnover of nematocysts was followed. *Coryphella verrucosa* selected a distinct nematocyst type from each of the 4 prey species used and the turnover of nematocysts was complete in 3 to 5 days.

It is postulated that nematocysts stored in cnidosac cells are turned over quickly because nematocysts have a finite viability as is suggested by Bode & Flick's (1976) studies on *Hydra*.

The selection of the nematocyst type by several species of nudibranchs eating the same coelenterate species and preference for different nematocyst types in different prey suggests that nudibranch-hydroid associations may be a useful model for studying the role of nematocysts both in coelenterates and in their nudibranch predators.

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